Urol Res (1999) 27:397–403

INVITED EDITORIAL

Inge Højgaard · Hans-Göran Tiselius

Crystallization in the nephron

Received: 24 December 1998 / Accepted: 11 March 1999

Abstract Recent experimental studies on the crystallization of calcium salts at different nephron levels support the theory that the initial formation of calcium concrements starts with an intratubular crystallization of calcium phosphate (CaP) and calcium oxalate (CaOx). CaP seems to be the initial crystallization product in pure CaP and mixed calcium phosphate–calcium oxalate (CaPCaOx) concrements, with the formation of CaP crystals at a nephron level above the collecting duct. Urinary macromolecules and cellular degradation products most probably promote this process. During the passage through the collecting duct, CaP might partly or completely dissolve at the lower pH encountered there. This might result in an increased concentration of calcium and hence an increased supersaturation with CaOx, which in turn can bring about a heterogeneous nucleation of CaOx on or around preformed CaP crystals or crystal aggregates. The final result will be mixed CaOxCaP or pure CaOx concrements. Pure CaOx concrements might also be the result of an initial CaOx crystallization at nephron levels above or in the collecting duct under conditions with a high urinary excretion of oxalate. Whether intratubular crystallization of calcium salts results in the formation of small harmless crystals excreted with urine or calcium stones appears to be determined by a complex process, involving kinetic factors that influence crystal growth and crystal aggregation and crystal retention.

Key words Calcium phosphate · Calcium oxalate · Nephron · pH · Urinary macromolecules

I. Højgaard (✉)
Department of Surgery, Västervik Hospital,
593 81 Västervik, Sweden

H.-G. Tiselius
Department of Urology, University Hospital,
141 86 Huddinge, Sweden

Introduction

Most urinary concrements are localized to the upper parts of the collecting system, and many concrements have signs of previous attachment to the calyx lining [15, 16, 76, 77]. This indicates that the formation of urinary concrements starts either in the calyceal space or at a nephron level. Bladder urine has a composition similar to that in calyces, although the concentration of some urinary macromolecules such as glycosaminoglycans is higher in bladder urine than in calyceal urine [24].

Bladder urine has a high inhibitory activity, a property that is obvious when whole voided urine is used in crystallization experiments, as excessive amounts of calcium or oxalate have to be added to start calcium oxalate (CaOx) crystallization within a reasonable period of time [8].

Crystalluria is a common finding in both recurrent stone formers and in normal subjects [25, 88], although recurrent stone formers excrete larger and more aggregated crystals than normal subjects [33, 78, 89, 103]. The common occurrence of crystalluria despite the appreciable inhibitory activity of whole urine makes it reasonable to assume that crystallization of calcium salts is initiated in the nephron, where the concentration of inhibitory macromolecules is much lower than in final urine.

Several reports in recent years have supported the theory that calcium salt crystallization is initiated within the nephron [5, 17, 20, 32, 46, 54, 59–61, 84], most probably as an intratubular event [44, 61]. There is no doubt, however, that crystal retention caused either by a trapping of crystal masses or by an interaction between crystals and cells is of great importance for the development of small harmless calcium crystals to urinary concrements [10–12, 46, 59, 66, 101, 102]. Knowledge of the crystallization of calcium salts in nephron urine has been obtained from in vitro experiments [5, 42, 61, 69, 96, 98] as well as in vivo experiments [32, 46, 49, 54].

In vitro studies

In 1978 Finlayson and Reid [27] claimed that the formation of a urolith in the upper urinary tract could not start as a free precipitated particle. In their calculations they used published data on tubular dimensions, crystalluria, urine flow and oxalate excretion. It was concluded that even under conditions at which all oxalate was allowed to precipitate at the fastest rate, the transit time of 3 min through the nephron was too short for CaOx crystals to grow to a size at which they would be trapped in the duct of Bellini.

In a recent publication, however, Kok and Khan [59] used new information and concluded from recalculations that it was possible to form CaOx crystals large enough to be trapped in the duct of Bellini. The difference between these contradictory results was mainly explained by crystal aggregation, which in contrast to crystal growth is a very fast process occurring in seconds. Other important issues were that variations in the tubular intraluminal diameter cause different flow velocities in different nephron segments, and the fact that the opening of the duct of Bellini at the papillary tip is not circular with a diameter of 200 μm, but rather split like: 7–23 μm wide and 60–120 μm long [50, 59]. Kok and Khan also considered the fact that nephrons had both short and long loops of Henle, with the majority being short. The transit time of urine through the nephron depends on the length of the loop of Henle, and the ion composition will differ between long and short loops. Furthermore attention was focused on variations in the urinary flow and the excretion of oxalate, which resulted in periods with peaks of CaOx supersaturation at high nephron levels.

Coe and coworkers [5, 17, 20] concluded from micropuncture data obtained in rats that the urine in the loop of Henle might be highly supersaturated with respect to calcium phosphate (CaP), whereas the collecting duct urine would be supersaturated with CaOx. In experiments in vitro with solutions with a composition corresponding to that of urine in the descending limb of the loop of Henle, precipitation of CaP was recorded under conditions with a high phosphate concentration.

Recently interesting data on the intratubular crystallization of calcium salts have been presented by Kok [60, 61]. On the basis of micropuncture studies in animals he extrapolated data to the ion composition at different levels of the human nephron. He concluded that CaP crystals could form during the transit of urine through the nephron segments above the collecting duct, with precipitation of CaP starting in the loop of Henle.

Literature data on urine composition at different nephron levels together with extrapolation from urine and blood concentrations of relevant urine constituents were used to derive approximate supersaturation levels in different part of the nephron [69]. The ion-activity products of CaOx (AP_{CaOx}), hydroxyapatite (AP_{HAP}) and brushite (AP_{Bru}) were calculated with the EQUIL2 program [104]. From these calculations it was concluded that the risk of CaP precipitation was high at levels above the collecting duct (CD; i.e. final urine), whereas in CD urine the risk was most pronounced for CaOx precipitation (Table 1). The ion composition in different parts of the nephron used in these calculations is summarized in Table 2 [42].

Important risk factors for the precipitation of CaOx and CaP are urinary pH and the excretion of calcium, oxalate, phosphate, citrate and magnesium [92–95]. These variables are subject to a considerable variation during the day and might differ from day to day and between different periods of the year [4, 91]. This normal variation might thus result in periods with a high risk of CaP precipitation as well as a high risk of CaOx precipitation. Inasmuch as a high excretion of calcium is a common finding in recurrent stone formers [80, 95], and the fact that also small changes in concentration of oxalate are considered an important risk factor in stone formation, we found it important to study how the crystallization of calcium salts at different nephron levels was affected by increased calcium and oxalate concentrations.

With an increased concentration of calcium, CaP was the initial precipitate in solutions with an ion composition corresponding to that in urine above the CD, and the highest relative risk of CaP precipitation was found in solutions simulating urine from the distal part of the

Table 1 The ion-activity products for calcium oxalate (*CaOx*), hydroxyapatite (*HAP*) and brushite (*Bru*) at different nephron levels

	AP_{CaOx} ($10^8 \times M^2$)	AP_{HAP} ($10^{48} \times M^9$)	AP_{Bru} ($10^7 \times M^2$)
Proximal tubule	0.103	34.0	0.87
Distal tubule, proximal part	0.102	0.023	0.46
Distal tubule, distal part	0.154	0.079	0.76
Collecting duct, end	1.95	0.366	4.21

Table 2 Ion composition of salt solutions corresponding to that of urine in different parts of the nephron

Concentration (mmol/l)	Proximal tubule	Distal tubule		Collecting duct
		Proximal part	Distal part	
Calcium	2.78	1.32	1.04	4.50
Magnesium	0.09	0.12	0.41	3.85
Phosphate	1.00	1.25	4.17	32.2
Oxalate	0.01	0.013	0.04	0.32
Citrate	0.08	0.10	0.35	3.21
Sodium	139	82	96	106
Potassium	3.20	0.95	22.5	63.7
Sulphate	3.30	4.20	13.8	20.8
pH	6.75	6.45	6.45	5.80

distal tubule [69]. In another study, approximate estimates of AP_{CaP} and AP_{CaOx} in distal tubular urine were derived by extrapolation from the 16-h urinary excretion of calcium, oxalate, citrate, magnesium and phosphate [97]. The pH was assumed to be 6.45 and others ions of importance for the ion-activity products were considered constant. The conclusion from these calculations was that the diurnal variation in urine composition and pH might result in peaks of CaP supersaturation in distal tubular urine, sufficiently high to result in CaP nucleation.

Solutions with a composition similar to that in the loop of Henle were not used in these experiments, but there is evidence that the risk for CaP precipitation is greatest in the thin descending limb of the loop of Henle [5, 17, 20, 60, 61]. Inasmuch as this segment is only permeable to water the urine at this level has an increased concentration of phosphate and calcium and a pH around 7.4.

Irrespective of the nephron level at which CaP first forms, the results from all experiments referred to indicate a high risk of CaP crystallization at nephron levels above the CD, particularly during periods with a high alkaline load and a high excretion of calcium and phosphate.

Although urine is supersaturated with CaP at nephron levels above the CD and with CaOx in the CD (Table 1), a homogeneous nucleation of calcium salts in urine is less likely. A homogeneous nucleation of CaOx is conceivable only in highly supersaturated and perfectly clean solutions without any impurities. Urine contains macromolecules, tubular cells, tubular membranes and various other types of cellular debris, which all might act as possible promoters for CaP as well as CaOx nucleation [22, 23, 31, 48, 49, 72, 73, 82, 83, 99, 106].

From the distal part of the distal nephron (DTd) to the end of the CD urine is mainly subjected to the reabsorption of water and a reduction in pH. To simulate this process experimentally, DTd solutions were evaporated in the presence and absence of dialysed bladder urine from normal men at various starting pH values [38]. At pH levels below 7.2 urinary macromolecules in dialysed urine apparently promoted CaP nucleation and counteracted the development of large CaP crystals [37, 38]. From these results it was not possible to conclude whether the smaller crystals formed in the presence of dialysed urine in solutions with a starting pH below 7.2 were the result of inhibition of crystal growth, inhibition of crystal aggregation or both. Inhibitory effects of urinary macromolecules on the growth of CaP crystals and aggregation of CaOx crystals have been reported by several authors [18, 21, 34, 36, 55, 57, 64, 81, 86–90, 105]. It has been claimed that an enhanced CaOx crystal aggregation is one of the most important factors that distinguishes stone formers from normal subjects [56, 58, 79, 85]. By spectrophotometric assessment of the sedimentation rate of hydroxyapatite (HAP) crystals in DTd-like solutions urinary macromolecules proved to be strong inhibitors of CaP crystal aggregation. This effect apparently was augmented by citrate in concentrations above 0.5 mmol/l [40]. In another experiment in which HAP seed crystals were added to salt solutions with an ion composition corresponding to that in the CD, scanning electron microscopic examination of the precipitate, formed as a result of volume reduction, disclosed smaller and less aggregated crystals in the presence of urinary macromolecules [39]. These studies suggest that urinary macromolecules have a complex effect on the initial crystallization of CaP and CaOx, promoting nucleation and inhibiting crystal growth and aggregation.

Most urinary calcium stones contain CaOx as the major component and CaP as a minor component [19, 95], and it is of note that CaP is often found in the core of the stone [14, 65, 75]. These findings indicate that CaP might be the initial crystal phase in mixed calcium stones, and a heterogeneous nucleation of CaOx induced by CaP has previously been reported by several authors [2, 7, 9, 29, 30, 62, 70, 71, 74, 88].

CaP formed in nephron segments above the CD might partly or completely dissolve during passage through the CD as a result of the lower pH encountered there [41, 61, 96, 98]. This dissolution may result in a substantially increased local concentration of calcium and hence an increased supersaturation with CaOx. Under these conditions heterogeneous nucleation of CaOx on or in the surroundings of dissolving CaP crystals might be possible (Fig. 1) [39, 41, 96, 98]. During the further passage through the CD these CaPCaOx crystal masses might increase in size by further growth and aggregation [42]. This process is enhanced by insufficient inhibition of growth and aggregation, which has been demonstrated by several authors [1, 18, 21, 26, 34, 35, 57, 58]. The ensuing large crystals might then be trapped at the tip of the papilla [59] or retained by crystal/cell interaction. Crystal/cell interaction might be affected by several urinary macromolecules that act either on the crystal surface or on the epithelial cell [66–68, 101] and it is reasonable to assume that under conditions with a low crystallization inhibitory power in urine, kinetic processes such as crystal growth, crystal aggregation and crystal/cell interaction will all be favoured [28, 67, 100, 101].

Whether the crystal mass is retained intratubularly or internalized by the tubular cells, a stone nidus will finally be localized to the tip of the papilla, where further growth and aggregation in the supersaturated calyceal urine might continue [50]. It can be expected that the complete dissolution of CaP crystals in the CD gives rise to pure CaOx crystals.

In vivo studies

While in vitro studies often focus on one aspect in calcium stone formation, in vivo studies consider the whole crystallization process. Most in vivo studies

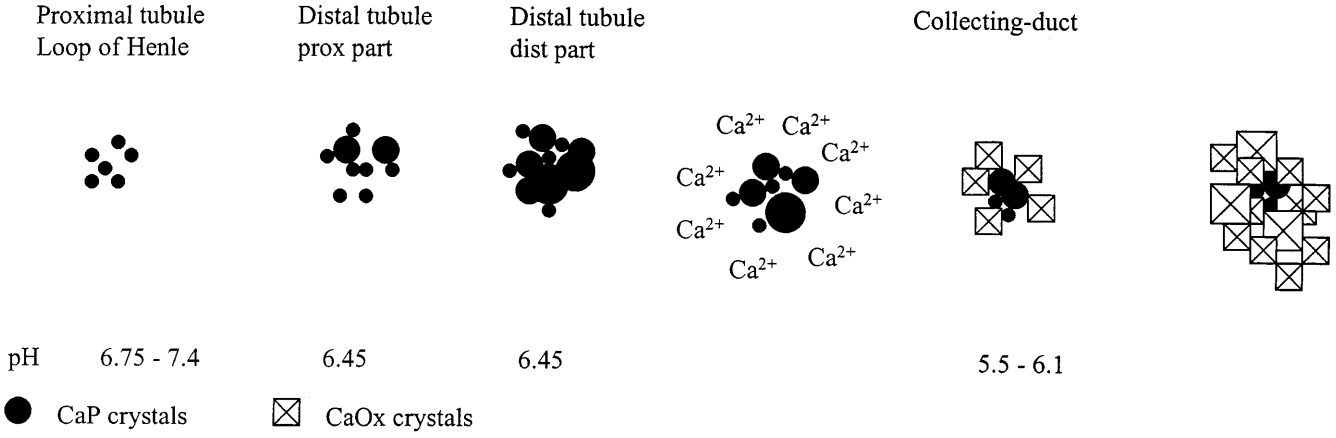

Fig. 1 Tentative model for the initial intratubular events in the formation of mixed calcium phosphate (Cap)–calcium oxalate (CaOx) concrements. The process is most probably modified by urinary macromolecules and cellular degradation products

concerning the crystallization of calcium stones have been carried out in rats. In these models hyperoxaluria has been induced by intraperitoneal or intravenous injections of oxalate [43, 46] or by a diet of ethylene glycol with or without ammonium chloride [10, 13, 51]. In these animals crystals were first identified intratubularly and later both intracellulary and interstitially [13, 50, 47]. De Bruijn and co-workers [13] found that although crystals were not present in the more proximal parts of the rat nephron after several days of recovery from a diet resulting in moderate hyperoxaluria, crystals were identified in the papillary region, both free in the calyceal space and subepithelially surrounded by interstitial cells. Khan and coworkers in numerous experiments studied the crystallization process in rats subjected to either acute or chronic hyperoxaluria [43, 45, 46, 51]. They showed that in acute hyperoxaluria CaOx crystals were initially recovered in the proximal tubules [46]. The same was found in animals with moderate and excessive hyperoxaluria, whereas in rats with mild hyperoxaluria, crystals initially were observed in the distal nephron segments [51]. It is, however, important to be aware of the fact that results in animal models cannot be directly extrapolated to the situation in humans. Firstly the hyperoxaluria induced often is unphysiological, with supersaturation levels probably not encountered in most idiopathic stone formers, even when the diurnal variations in calcium and oxalate excretion are considered. Another important issue is that the high oxalate concentration counteracts an initial CaP crystallization. Nevertheless experimental in vivo models have increased our understanding of the initial event in calcium salt crystallization, and the animal experiments also support the idea that the crystallization process starts as an intratubular event and that crystal retention is an extremely important factor for the development of urinary concrements.

Conclusion

Intratubular urine is supersaturated with respect to CaP, CaOx or both, with supersaturation levels favouring the precipitation of CaP in urine at nephron levels above the CD and CaOx in the CD [5, 17, 20, 42, 52, 60, 61, 69, 96, 98]. Urinary macromolecules such as Tamm-Horsfall protein, nephrocalcin, uropontin, urinary prothrombin fragment 1, glycosaminoglycans and inter-α-trypsin inhibitor as well as low molecular weight substances such as citrate, magnesium and pyrophosphate are probably of great importance as determinants of the intratubular development of CaP and CaOx crystals. It is reasonable to assume that under certain conditions, CaP formed at a high nephron level might partly or completely dissolve in the CD when the pH is low [41, 42, 61, 98]. This might result in an increased local concentration of calcium and hence an increased supersaturation with CaOx. This makes CaOx precipitation on or adjacent to the preformed CaP crystals possible. Whether the CaP crystal mass causes a heterogeneous nucleation of CaOx on the CaP surface, or the nucleation of CaOx in the highly supersaturated urine is promoted by urinary macromolecules or other factors attached to the CaP crystal mass, is not known. In vitro studies have shown clearly that CaP can induce heterogeneous nucleation of CaOx [3, 63, 70] and in a recent paper by Khan [53] it was postulated that the heterogeneous nucleation of CaOx most probably was induced by urinary macromolecules and cellular degradation products, inasmuch as these substances were intimately associated with CaP crystals. Whatever the final mechanism might be, CaP formed at high nephron levels is obviously of importance for the subsequent formation of CaOx in the CD. Probably only under conditions with a very high urinary excretion of oxalate will CaOx be the primary crystal type at nephron levels above the CD [59, 61, 69].

From the results discussed in this review it appears important that the initial crystallization events are studied under conditions simulating those in the nephron, whereas studies on the final steps in the development of a stone most appropriately should be made in whole urine. In experiments with whole urine it is important to be

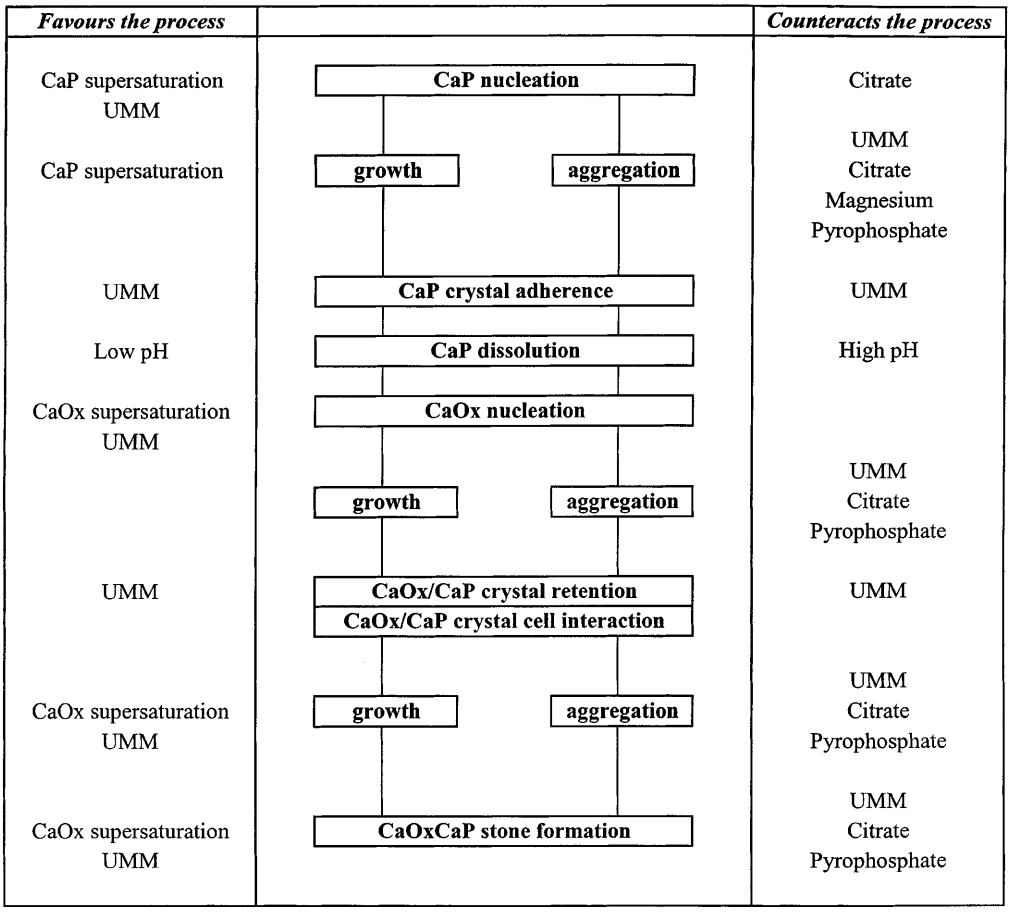

Fig. 2 Some factors of importance for the development of a calcium renal stone. *UMM* urinary macromolecules

aware of the alterations in composition that might result from the preparation technique [6].

An overview of the different mechanisms that might contribute to the formation of calcium stones in the renal tract is given in Fig. 2.

References

1. Achilles W, Dekanic D, Burk M, Schalk CH, Tucak A, Karner I (1991) Crystal growth of calcium oxalate in urine of stone-formers and normal controls. Urol Res 19:159
2. Achilles W, Jöckel U, Schaper A, Ulshöfer B, Riedmiller H (1994) Formation of urinary stones in vitro: growth of calcium oxalate on spherulites of calcium phosphate in gel: In: Ryall RL, Bais R, Marshall VR, Rofe AM, Smith LH, Walker VR (eds) Urolithiasis 2. Plenum Press, New York, p 161
3. Achilles W, Jockel U, Scharper A, Burk B, Reidmiller H (1995) In vitro formation of "urinary stones": generation of spherulites of calcium phosphate in gel and overgrowth with calcium oxalate using a new flow model of crystallization. Scanning Microsc 9:577
4. Ahlstrand C, Larsson L, Tiselius HG (1984) Variations in urine composition during the day in patients with calcium oxalate stone disease. J Urol 131:77
5. Asplin JR, Mandel NS, Coe FL (1996) Evidence of calcium phosphate supersaturation in the loop of Henle. Am J Physiol 270:F604
6. Atmani F, Glenton PA, Khan SR (1998) Identification of proteins extracted from calcium oxalate and calcium phosphate crystals induced in urine of healthy and stone forming subjects. Urol Res 26:201
7. Baumann JM (1985) Can the formation of calcium oxalate stones be explained by crystallization processes in urine? Urol Res 13:267
8. Baumann JM (1995) Methods for crystallization studies: introduction. In: Rao PN, Kavanagh JP, Tiselius HG (eds) Urolithiasis: consensus and controversies. Manchester, p 57
9. Berg C, Tiselius HG (1989) The effects of citrate on hydroxyapatite induced calcium oxalate crystallization and on the formation of calcium phosphate crystals. Urol Res 17:167
10. Boevé ER, Ketelaars GAM, Vermeij M, Cao LC, Schröder FH, de Bruijn WC (1993) An ultrastructural study of experimentally induced microliths in rat proximal and distal tubules. J Urol 149:893
11. Boevé ER, Cao LC, Verkoelen CF, Romijn JC, de Bruijn WC, Schröder FH (1994) Glycosaminoglycans and other sulphated polysaccharides in calculogenesis of urinary stones. World J Urol 12:43
12. Bruijn WC de, Boevé ER, van Run PRWA, van Miert PPMC, Romijn JC, Verkoelen CF, Cao LC, Schröder FH (1994) Etiology of experimental calcium oxalate monohydrate nephrolithiasis in rats. Scanning Microsc 8:541
13. Bruijn WC de, Boevé ER, van Run PRWA, van Miert PPMC, de Water R, Romijn JC, Verkoelen CF, Cao LC, Schröder FH (1995) Etiology of calcium oxalate nephrolithiasis in rats. I. Can this be a model for human stone formation? Scanning Microsc 9:103
14. Chambers A, Hodgkinson A, Hornung G (1972) Electron probe analysis of small urinary tract calculi. Invest Urol 9:376
15. Cifuentes Delatte L, Minon-Cifuentes J, Medina JA (1985) Papillary stones: calcified renal tubules in Randall's plaques. J Urol 133:490
16. Cifuentes Delatte L, Minon-Cifuentes J, Medina JA (1987) New studies on papillary calculi. J Urol 137:1024

17. Coe Fl, Parks JH (1990) Defences of an unstable compromise: crystallization inhibitors and the kidney's role in mineral regulation. Kidney Int 38:625
18. Coe FL, Parks JH, Nakagawa Y (1991) Protein inhibitors of crystallization. Semin Nephrol 11:98
19. Daudon M, Donsimoni R, Hennequin C, Fellahi S, Moel G Le, Paris M, Troupel S, Lacour B (1995) Sex- and age-related composition of 10 617 calculi analyzed by infrared spectroscopy. Urol Res 23:319
20. DeGanello S, Asplin J, Coe FL (1990) Evidence that tubule fluid in the thin segment of the loop of Henle normally is supersaturated and forms a poorly crystallized hydroxyapatite that can initiate renal stones. Kidney Int 37:472
21. Doyle LR, Marshall VR, Dawson CJ, Ryall RL (1995) Calcium oxalate crystal matrix extract: the most potent macromolecular inhibitor of crystal growth and aggregation yet tested in undiluted human urine in vitro. Urol Res 23:53
22. Drach GW, Thorson S, Randolph A (1980) Effects of urinary organic macromolecules on crystallization of calcium oxalate: enhancement of nucleation. J Urol 123:519
23. Drach GW, Sarig S, Randolph AD, Torson S (1982) The paradox of inhibition and enhancement of the formation of urinary stones. Urol Res 10:165
24. Edyvane KA, Ryall RL, Marshall VR (1984) The contribution of bladder secretions to the crystal growth inhibitor activity of urine. In: Ryall RL, Brockis JG, Marshall VR, Finlayson B (eds) Urinary stone Churchill Livingstone, New York, p 198
25. Elliott JS, Rabinowitz IN (1976) Calcium oxalate crystalluria. J Urol 116:773
26. Erwin DT, Kok DJ, Alam J, Vaughn J, Coker O, Carriere BT, Lindberg J, Husseri FE, Fuselier H, Cole FE (1994) Calcium oxalate agglomeration reflects stone-forming activity: citrate inhibition depends on macromolecules larger than 30 kilodalton. Am J Kidney Dis 24:893
27. Finlayson B, Reid F (1978) The expectation of free and fixed particles in urinary stone disease. Invest Urol 15:442
28. Gill WB, Jones KW, Ruggiero KJ (1982) Protective effects of heparin and other sulfated glycosaminoglycans on crystal adhesion to injured urothelium. J Urol 127:152
29. Grases F, Conte A, Gil JJ (1988) Simple method for the study of heterogeneous nucleation in calcium oxalate urolithiasis. Br J Urol 61:468
30. Grases F, Conte A (1992) Urolithiasis, inhibitors and promoters. Urol Res 20:86
31. Grases F, Garcia-Ferragut L, Costa-Bauza A (1996) Study of the early stages of renal stone formation: experimental model using urothelium of pig urinary bladder. Urol Res 24:305
32. Hering F, Lueoend G, Briellmann T, Seiler H, Guggenheim H, Rutishauser G (1988) Stone formation in the human kidney. In Walker VR, Sutton RAL, Cameron ECB, Pak CYC, Robertson WG (eds) Urolithiasis. Plenum Press, New York, p 73
33. Herrmann U, Schwille PO, Kuch P (1991) Crystalluria determined by polarization microscopy: technique and results in healthy control subjects and patients with idiopathic recurrent calcium urolithiasis classified in accordance with calciuria. Urol Res 19:151
34. Hess B, Nakagawa Y, Coe FL (1989) Inhibition of calcium oxalate monohydrate crystal aggregation by urine proteins. Am J Physiol 257:F99
35. Hess B, Zipperle L, Jaeger P (1993) Citrate and calcium effects on Tamm-Horsfall glycoprotein as a modifier of calcium oxalate crystal aggregation. Am J Physiol 265:F784
36. Hess B, Meinhardt U, Zipperle L, Giovanoli R, Jaeger P (1995) Simultaneous measurements of calcium oxalate crystal nucleation and aggregation: impact of various modifiers. Urol Res 23:231
37. Højgaard I, Fornander AM, Nilsson MA, Tiselius HG (1996) Crystallization during volume reduction of solutions with an ion-composition corresponding to that in the distal tubuli. Scanning Microsc 10:487
38. Højgaard I, Fornander AM, Nilsson MA, Tiselius HG (1999) The effect of pH changes on the crystallization of calcium salts in solutions with an ion composition corresponding to that in the distal tubule. Urol Res, in press
39. Højgaard I, Fornander AM, Nilsson MA, Tiselius HG (1999) Crystallization during volume reduction of solutions with a composition corresponding to that in the collecting duct: the influence of hydroxyapatite seed crystals and urinary macromolecules. Urol Res, in press
40. Højgaard I, Tiselius HG (1998) The effects of citrate and urinary macromolecules on the aggregation of hydroxyapatite crystals in solutions with a composition similar to that in the distal tubule. Urol Res 26:89
41. Højgaard I, Fornander AM, Nilsson MA, Tiselius HG (1998) The influence of hydroxyapatite seed on the crystallization induced by volume reduction of solutions with an ion-composition corresponding to the distal tubule at different pH levels. Scand J Urol Nephrol 32:311
42. Højgaard I (1998) Crystallisation of calcium phosphate and calcium oxalate in solutions simulating the composition at different levels of the nephron. PhD. thesis, University of Linköping, Sweden
43. Khan SR, Finlayson B, Hackett RL (1979) Histologic study of the early events in oxalate induced intranephrotic calculosis. Invest Urol 17:199
44. Khan SR, Hackett RL (1980) The use of SEM in the study of oxalate induced experimental nephrolithiasis. Scanning Microsc 3:379
45. Khan SR (1991) Pathogenesis of oxalate urolithiasis: lessons from experimental studies with rats. Am J Kidney Dis 17:398
46. Khan SR, Hackett RL (1991) Retention of calcium oxalate crystals in renal tubules. Scanning Microsc 5:707
47. Khan SR, Shevock PN, Hackett RL (1992) Acute hyperoxaluria, renal injury and calcium oxalate urolithiasis. J Urol 147:226
48. Khan SR, Whalen PO, Glenton PA (1993) Heterogeneous nucleation of calcium oxalate in the presence of membrane vesicles. J Crystal Growth 134:211
49. Khan SR, Atmani F, Glenton PA, Hou Z, Talham DR, Khursshid M (1995) Lipids and membranes in the organic matrix of urinary calcidic crystals and stones. Calcif Tissue Int 59:357
50. Khan SR (1995) Experimental calcium oxalate nephrolithiasis and the formation of human urinary stones. Scanning Microsc 9:89
51. Khan SR (1995) Calcium oxalate crystal interaction with renal tubular epithelium, mechanism of crystal adhesion and its impact on stone development. Urol Res 23:71
52. Khan SR, Glenton PA (1995) Deposition of calcium phosphate and calcium oxalate crystals in the kidneys. J Urol 153:811
53. Khan SR (1997) Calcium phosphate/calcium oxalate crystal association in urinary stones: implications for heterogeneous nucleation of calcium oxalate. J Urol 157:376
54. Kohri K, Kodama M, Ishikawa Y, Ktayama Y, Matsuda H (1991) Immunofluorescent study of the interaction between collagen and calcium oxalate crystals in renal tubules. Eur Urol, 19:249
55. Koide T, Takemoto M, Itatani H, Takaha M, Sonoda T (1981) Urinary macromolecular substances as natural inhibitors of calcium oxalate crystal aggregation. Invest Urol 18:382
56. Kok DJ, Papapoulos SE, Bijvoet OLM (1986) Excessive crystal agglomeration with low citrate excretion in recurrent stone formers. Lancet 1:1056
57. Kok DJ, Papapoulos SE, Blomen LJMJ, Bijvoet OLM (1988) Modulation of calcium oxalate monohydrate crystallization kinetics in vitro. Kidney Int 34:346
58. Kok DJ, Papapoulos SE, Bijvoet OLM (1990) Crystal agglomeration is a major element in calcium oxalate urinary stone formation. Kidney Int 37:51
59. Kok DJ, Khan SR (1994) Calcium oxalate nephrolithiasis, a free or fixed particle disease. Kidney Int 46:847

60. Kok DJ (1995) Intratubular crystallization events. In: Tiseliùs HG (ed) Renal stones: aspects of their formation, removal and prevention. Proceedings of the Sixth European Symposium on Urolithiasis. Akademitryck, Edsbruk, Sweden, p 26
61. Kok DJ (1997) Intratubular crystallization events. World J Urol 15:219
62. Koutsoukos PG, Nancollas GH (1981) Crystal growth of calcium phosphates: epitaxial considerations. J Crystal Growth 53:10
63. Koutsoukos PG, Sheehanm ME, Nancollas GH (1981) Epitaxial considerations in stone formation. II. The oxalate-phosphate system. Invest Urol 18:358
64. Lanzalaco AC, Sheehan ME, White DJ, Nancollas GH (1982) The mineralization inhibitory potential of urines: a constant composition approach. J Urol 128:845
65. Leusmann DB, Entrup M, Schmandt W, Blaschke R (1984) Results combined phase and texture analysis of 1028 urinary concrements. Urol Res 12:94
66. Lieske JC, Toback FG (1993) Regulation of renal epithelial cell endocytosis of calcium oxalate monhydrate crystals. Am J Physiol 264:F800
67. Lieske JC, Leonard R, Toback FG (1995) Adhesion of calcium oxalate monohydrate crystals to renal epithelial cells inhibited by specific anions. Am J Physiol 268:F604
68. Lieske JC, Norris R, Toback FG (1997) Adhesion of hydroxyapatite crystals to anionic sites on the surface of renal epithelial cells. Am J Physiol 273:F224
69. Lupták J, Bek-Jensen H, Fornander AM, Højgaard I, Nilsson AM, Tiselius HG (1994) Crystallization of calcium oxalate and calcium phosphate at supersaturation levels corresponding to those in different parts of the nephron. Scanning Microsc 8:47
70. Meyer JL, Bergert JH, Smith LH (1975) Epitaxial relationships in urolithiasis: the calcium oxalate monohydrate-hydroxyapatite system. Clin Sci Mol Med 49:369
71. Nancollas GH (1983) Crystallization theory relating to urinary stone formation. World J Urol 1:131
72. Nishio S, Kavanagh JP, Faragher EB, Garside J, Blacklock NJ (1990) Calcium oxalate crystallisation kinetics and the effects of calcium gamma-carboxyglutamic acid. Br J Urol 66:351
73. Pak CYC, Ruskin B (1970) Calcification of collagen by urine in vitro: dependence on the degree of saturation of urine with respect to brushite. J Clin Invest 49:2353
74. Pak CYC, Hayashi Y, Arnold LH (1976) Heterogeneous nucleation with urate, calcium phosphate and calcium oxalate. Proc Soc Exp Biol Med 153:83
75. Prien L (1955) Studies on urolithiasis. III. Physicochemical principles in stone formation and prevention. J Urol 73:627
76. Prien EL (1975) The riddle of Randall's plaques. J Urol 114:500
77. Randall A (1937) The origin and growth of renal calculi. Ann Surg 105:1009
78. Robertson WG, Peacock M, Nordin BEC (1969) Calcium crystalluria in recurrent renal stone formers. Lancet II:21
79. Robertson WG, Peacock M (1972) Calcium oxalate crystalluria and inhibitors of crystallization in recurrent renal stone-formers. Clin Sci 43:499
80. Robertson WG, Peacock M, Heyburn PJ, Marshall DH, Clark PB (1978) Risk factors in calcium stone disease of the urinary tract. Br J Urol 50:449
81. Robertson WG, Scurr DS, Bridge CM (1981) Factors influencing the crystallization of calcium oxalate in urine: critique. J Crystal Growth 53:182–194
82. Rose GA, Sulaiman S (1982) Tamm-Horsfall mucoproteins promote calcium oxalate crystal formation in urine: quantitative studies. J Urol 127:177
83. Rose GA, Sulaiman S (1984) Tamm-Horsfall mucoprotein promotes calcium phosphate crystal formation in whole urine: quantitative studies. Urol Res 12:217
84. Rushton HG, Spector M, Rodergs AL, Hughson M, Magura CE (1981) Developmental aspects of calcium oxalate tubular deposits and calculi induced in rat kidneys. Invest Urol 19:52
85. Ryall RL, Harnett RM, Hibberd CM, Edyvane KA, Marshall VR (1989) The effect of macromolecules on the crystallization of calcium oxalate in human urine. In: Walker VR, Sutton RAL, Cameron ECB, Pak CYC, Robertson WG (eds). Urolithiasis Plenum Press, New York, p 133
86. Ryall RL, Harnett RM, Hibberd CM, Edyvane KA, Marshall VR (1991) Effects of chondroitin sulphate, human serum albumin and Tamm-Horsfall mucoprotein on calcium oxalate crystallization in undiluted human urine. Urol Res 19:181
87. Ryall RL, Grover PK, Stapleton AMF, Barrel DK, Tang Y, Moritz RL, Simpson RJ (1995) The urinary F1 activation peptide of human prothrombin is a potent inhibitor of calcium oxalate crystallization in undiluted urine in vitro. Clin Sci 89:533
88. Smith LH, Werness PG (1983) Hydroxyapatite: the forgotten crystal in calcium urolithiasis. Trans Am Clin Climatol Assoc 95:183
89. Springmann KE, Drach GW, Gottung B, Randolph AD (1986) Effects of human urine on aggregation of calcium oxalate crystals. J Urol 135:69
90. Suzuki K, Ryall RL (1996) The effect of heparan sulphate on the crystallization of calcium oxalate in undiluted, ultrafiltrated human urine. Br J Urol 78:15
91. Tiselius HG, Almgård LE (1977) The diurnal excretion of oxalate and the effect of pyrodoxine and ascorbate on oxalate excretion. Eur Urol 3:41
92. Tiselius HG (1982) An improved method for the routine biochemical evaluation of patients with recurrent calcium oxalate stone disease. Clin Chim Acta 122:409
93. Tiselius HG (1984) A simplified estimate of the ion-activity product of calcium phosphate in urine. Eur Urol 10:191
94. Tiselius HG (1987) Measurement of the risk of calcium phosphate crystallization in urine. Urol Res 15:79
95. Tiselius HG (1996) Solution chemistry of supersaturation. In: Coe FL, Favus MJ, Pak CYC, Parks JH, Preminger GM (eds) Kidney stones: medical and surgical management. Lippincott-Raven, Philadelphia New York, p 33
96. Tiselius HG, Højgaard I, Fornander AM, Nilsson MA (1996) Is calcium phosphate the natural promoter of calcium oxalate crystallization. In: Pak CYC, Resnick MI, Preminger GM (eds) Urolithiasis. Proceedings of the Eighth International Symposium on Urolithiasis. Dallas, TX, p 238
97. Tiselius HG, Fornander AM, Nilsson MA (1997) Estimated levels of supersaturation with calcium phosphate and calcium oxalate in the distal tubule. Urol Res 25:153
98. Tiselius HG, Fornander AM, Nilsson MA (1998) Studies on the crystallization process following addition of calcium phosphate crystals to solutions with a composition corresponding to that in the collecting duct. Scanning Microsc, in press
99. Tozuka K, Yonese Y, Konjiki T, Sudo T (1987) Study of calcium phosphate crystalluria. J Urol 138:326
100. Verkoelen CF, Romijn JC (1996) Oxalate transport and calcium oxalate renal stone disease. Urol Res 24:183
101. Verkoelen CF, Romijn JC, Cao LC, Boevé ER, de Bruijn WC, Schröder FH (1996) Crystal-cell interaction inhibition by polysaccharides. J Urol 155:749
102. Verkoelen CF. Cell cultures and nephrolithiasis. World J Urol 15:229
103. Werness PG, Bergert JH, Smith LH (1981) Crystalluria. J Crystal Growth 53:161
104. Werness PG, Brown CM, Smith LH, Finlayson B (1985) EQUIL2: a BASIC computer program for the calculation of urinary saturation. J Urol 134:1242
105. Yamaguchi S, Yoshioka T, Utsunomiya M, Koide T, Osafune M, Okuyama A, Sonoda T (1993) Heparan sulfate in stone matrix and its inhibitory effect on calcium oxalate crystallization. Urol Res 21:187
106. Yoshioka T, Koide T, Utsunomiya M, Itatani H, Oka T, Sonoda T (1989) Possible role of Tamm-Horsfall glycoprotein in calcium oxalate crystallisation. Br J Urol 64:463